

# **PAPER – II PLANT DIVERSITY - I (PHYCOLOGY AND BRYOLOGY)**

**Subject Code: 18MBO12C**

## **Methods of cultivation of fresh water and marine Algae**

### **Algal production in outdoor ponds**

Large outdoor ponds either with a natural bottom or lined with cement, polyethylene or PVC sheets have been used successfully for algal production. The nutrient medium for outdoor cultures is based on that used indoors, but agricultural-grade fertilizers are used instead of laboratory-grade reagents (Table 2.5). However, fertilization of mass algal cultures in estuarine ponds and closed lagoons used for bivalve nurseries was not found to be desirable since fertilizers were expensive and it induced fluctuating algal blooms, consisting of production peaks followed by total algal crashes. By contrast, natural blooms are maintained at a reasonable cell density throughout the year and the ponds are flushed with oceanic water whenever necessary. Culture depths are typically 0.25-1 m. Cultures from indoor production may serve as inoculum for monospecific cultures. Alternatively, a phytoplankton bloom may be induced in seawater from which all zooplankton has been removed by sand filtration. Algal production in outdoor ponds is relatively inexpensive, but is only suitable for a few, fast-growing species due to problems with contamination by predators, parasites and “weed” species of algae. Furthermore, outdoor production is often characterized by a poor batch to batch consistency and

unpredictable culture crashes caused by changes in weather, sunlight or water quality.

Mass algal cultures in outdoor ponds are commonly applied in Taiwanese shrimp hatcheries where *Skeletonema costatum* is produced successfully in rectangular outdoor concrete ponds of 10-40 tons of water volume and a water depth of 1.5-2 m.

### **Culture of sessile micro-algae**

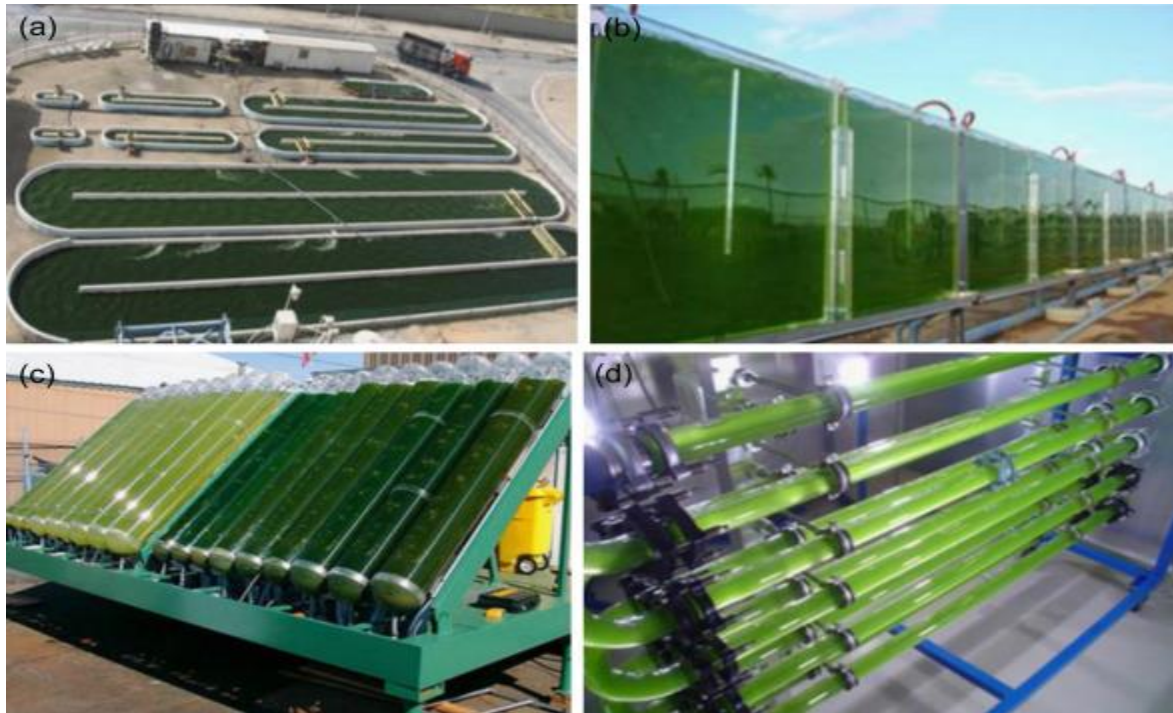
Farmers of abalone (*Haliotis* sp.) have developed special techniques to provide food for the juvenile stages which feed in nature by scraping coralline algae and slime off the surface of rocks using their radulae. In culture operations, sessile micro-algae are grown on plates of corrugated roofing plastic, which serve as a substrate for the settlement of abalone larvae. After metamorphosis, the spat graze on the micro-algae until they become large enough to feed on macro-algae. The most common species of micro-algae used on the feeder plates are pennate diatoms (e.g. *Nitzschia*, *Navicula*). The plates are inoculated by placing them in a current of sand filtered seawater. Depending on local conditions, the micro-algae cultures on the plates take between one and three weeks to grow to a density suitable for settling of the larvae. As the spat grow, their consumption rate increases and becomes greater than the natural production of the micro-algae. At this stage, the animals are too fragile to be transferred to another plate and algal growth may be enhanced by increasing illumination intensity and/or by the addition of fertilizer.

### **Quantifying algal biomass**

There are several ways to evaluate the quantity of algal biomass present in cultures either by counting the number of cells or through determination of volume, optical density or weight.

Cells can be counted either with an electronic particle counter or directly under a microscope, using a haematocytometer. The Coulter<sup>®</sup> counter and similar instruments need appropriate calibration for each algal species to be counted. Detailed instructions on operation of electronic cell counting can be found in Sheldon and Parsons (1967). The presence of contaminating particles in the same size range as the algae and failure of cells to separate after cell division may be possible sources of erroneous counts. Counting with a microscope has the advantage of allowing control of the quality of the cultures. The major difficulty in microscopic counts is reproducibility, which is a function of the sampling, diluting, and filling of the counting chamber, as well as the choice of the right type of counting chamber and range of cell concentration. Counting chambers, recommended for various cell sizes and concentrations, are listed in Table 2.9. Worksheet 2.2. details on the operation of two types of counting chambers, namely Fuchs-Rosenthal and Bürker.

A relationship between optical density and cellular concentration can be established using a spectrometer. However, variations may occur due to the fact that the chlorophyll concentration in the algal cell varies according to the culture conditions and therefore affects this relationship. In this way, a culture under low lighting conditions will be comparatively more pigmented and will eventually result in higher readings for optical density.



## 2. CULTURE CONDITIONS NECESSARY FOR GROWTH OF MARINE ALGAE

### 2.1 Light

This is normally provided by fluorescent lamps. The most commonly used types are 'cool-white' or 'daylight'. Increasing the light intensity usually means better growth and faster division of algal cells and, therefore, the production of more food. It is important to make the most efficient use of the artificial light as lamps also generate heat and may make the culture too hot. The indoor culture systems described in Section 5 of this leaflet are all designed to use light efficiently. Cultures of algae can also be grown outdoors, using natural daylight, and examples of this method are also given in Section 5.

### 2.2 Heat

Most types of algae grow well at temperatures from 17°C to 22°C. Lower temperatures will not usually kill the algae, but will reduce their growth rate. Above 27°C, most types of algae will die. If necessary, cultures can be cooled by a flow of cold water over the surface of the culture vessel or by controlling the air temperature with refrigerated air conditioning units.

### 2.3 Nutrients

Nutrients are the inorganic salts required for plant growth. For algae culture, it is convenient to make up strong standard solutions which, in appropriate dilutions in sea water, provide the culture medium. Recipes for the nutrient salt solutions, used for culture of algae at MAFF's Fisheries Laboratory at Conwy are given in Table 2.

## 2.4 Mixing

By mixing, the necessary light and nutrients become available to all of the cells. Algae cultures are usually mixed by bubbling air through them. This can be supplied from a compressor or via an air blower, and will also act as a carrier gas for carbon dioxide. Cultures may also be mixed by mechanical means using, for example, stirrers.

## 2.5 Carbon dioxide

Providing the algae with extra carbon, in the form of the gas carbon dioxide ( $\text{CO}_2$ ), will give much faster growth.  $\text{CO}_2$  is supplied from compressed gas cylinders, and only a very little is needed (about half of one percent) in the air supplied to the culture. The  $\text{CO}_2$  should be passed through a flow meter to ensure that the amount used will keep the pH of the culture between 7.8 and 8.0. The pH can be checked with indicator papers, which change colour with a change in pH, or with a pH meter. Both the air and the  $\text{CO}_2$  should be filtered through an in-line filter unit of 0.3 microns to 0.5 microns before entering the culture, as this helps to prevent other, possibly contaminating, organisms from getting into the cultures.

## 2.6 Salinity

Salinities of between 25 and 30 psu (practical salinity units) (UNESCO, 1981)\* are generally best for the culture of flagellates, and between 20 and 25 psu for the culture of diatoms. These salinities can be obtained by diluting sea water with tap water. Salinity can be measured with an hydrometer or a refractometer.

**Table 2. Nutrient salt solutions**

Constituents	Quantities
<b>Solution A</b>	
Ferric chloride ( $\text{FeCl}_3$ )	0.8 g <sup>(a)</sup>
Manganous chloride ( $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ )	0.4 g
Boric acid ( $\text{H}_3\text{BO}_3$ )	33.6 g
EDTA <sup>(b)</sup> , di-sodium salt	45.0 g
Sodium di-hydrogen orthophosphate ( $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ )	20.0 g
Sodium nitrate ( $\text{NaNO}_3$ )	100.0 g
Solution B	1.0 ml
Make up to 1 litre with fresh water <sup>(c)</sup>	Heat to dissolve
<b>Solution B</b>	
Zinc chloride ( $\text{ZnCl}_2$ )	2.1 g
Cobaltous chloride ( $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ )	2.0 g
Ammonium molybdate ( $(\text{NH}_4)_6\text{MO}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ )	0.9 g
Cupric sulphate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ )	2.0 g
Concentrated HCl	10.0 ml
Make up to 100 ml with fresh water <sup>(c)</sup>	Heat to dissolve

### **Solution C**

Vitamin B <sub>1</sub>	0.2g
Solution E	25.0ml
Make up to 200 ml with fresh water <sup>(e)</sup>	

### **Solution D (for culture of all diatoms— used in addition to solutions A and C)**

Sodium metasilicate (Na <sub>2</sub> SiO <sub>3</sub> ·5H <sub>2</sub> O)	40.0 g
Make up to 1 litre with fresh water <sup>(e)</sup>	Shake to dissolve

### **Solution E**

Vitamin B <sub>12</sub>	0.1 g
Make up to 250 ml with fresh water <sup>(e)</sup>	

### **Solution F (for culture of *Chroomonas salina*— used in addition to solutions A and C)**

Sodium nitrate (NaNO <sub>3</sub> )	200.0 g
Make up to 1 litre with fresh water <sup>(e)</sup>	(add 1 ml per litre of culture)

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## **2.7 Cleanliness**

The sea water containing the algae must be clean or unwanted types of algae and other contaminants, which may feed on or compete with the algae, will grow in the cultures.

Small amounts (of up to about 10 litres) of sea water can be autoclaved (sterilised by steam at a high pressure — a pressure cooker is a small autoclave), or pasteurised (heated to 80°C for 1-2 hours, cooling to room temperature for at least 18 hours, then reheated to 80°C for a further 1-2 hours) or boiled. Using these methods, a container of suitable material (e.g. borosilicate glass (Pyrex)), can be treated at the same time.

Larger volumes of sea water can be cleansed by filtration. There is a wide range of suitable equipment commercially available for this purpose, which includes cartridge filters, filter assemblies using diatomaceous earth as an aid to efficient filtration, and 'swimming pool type' filters. The main consideration is that the equipment chosen can cope with the volume of sea water that is needed. Filtration to remove particles greater than 2 microns is essential, and the removal of particles larger than half a micron is desirable. A large quantity of sea water can also be cleansed by passing it slowly through an ultra-violet light sterilising unit following filtration to remove particles greater than 2 microns in diameter.

### **3. GENERAL MAINTENANCE OF ALGAE CULTURES**

#### **3.1 Stock culture**

All algae culture systems require a set of 'stock' cultures, usually of about 250 ml in volume, to provide the reservoir of algal cells from which to start the larger-scale cultures which will be used for feeding.

Stock cultures are kept in small flasks, such as 500 ml borosilicate glass, flat-bottomed boiling flasks fitted with cotton-wool bungs. Two types of culture medium can be used:

- (a) Erdschreiber culture medium, which is difficult to prepare (Table 3(a)), but very reliable; and
- (b) a simpler, but less reliable culture medium, the preparation of which is described in Table 3(b).

#### **3.2 Sub-culture**

Stock cultures must be sub-cultured frequently (preferably weekly). Sub-culturing involves inoculating some cells from an old stock culture into fresh culture medium, so that the cells can continue to grow and divide and remain healthy. If sub-culturing is not carried out, the algal cells in the stock culture will eventually die. It is important to take precautions to prevent contaminants from the air entering the stock cultures when sub-culturing.

To start a new stock culture, about 20 ml of algae are taken from a stock culture which has been growing for 6 to 7 days and poured into a flask containing 250 ml of fresh culture medium. After removing the cotton-wool bungs, and before and after pouring, the necks of both flasks should be passed through a gas flame, such as that from a Bunsen burner or spirit lamp, to kill any surface and airborne contaminants, such as bacteria, that might enter the culture. To grow, the new stock culture should be put about 20 cm from a fluorescent lamp that is lit continually. The air temperature around the culture should be less than 25°C. Note that stock cultures do not require an air/CO<sub>2</sub> supply.

After sub-culturing, the remainder of the old stock culture can be used to start a batch culture of up to 10 litres. This method is described more fully in Section 5. If the stock culture is not required immediately, it may be kept for up to 3 weeks on a shelf in a north-facing window (away from direct sunlight), but after this time it should be discarded.

**Table 3(a). The preparation of Erdschreiber medium**

Constituents	Quantities
(a) Unfiltered sea water	2 litres
(b) Soil (woodland or pasture — no insecticides or fertilisers)	1 kg
(i) Mix with freshwater	1 litre
(ii) Autoclave	60 min at 1.06 kg per square cm (15 psi)
(iii) Decant	—
(iv) Filter	Whatman no. 1 paper, <i>then</i> glass-fibre (GF/C) paper
(v) Store in deep freeze	
(c) Nitrate/phosphate solution, obtained by dissolving:	
(i) Sodium nitrate ( $\text{NaNO}_3$ )	40 g
<i>and</i>	
(ii) Di-sodium hydrogen phosphate ( $\text{Na}_2\text{HPO}_4$ )	4 g
<i>in</i>	
(iii) Distilled water	200ml
(d) Silicate stock solution, obtained by dissolving:	
(i) Sodium metasilicate ( $\text{Na}_2\text{SiO}_3 \cdot 5\text{H}_2\text{O}$ )	8 g
<i>in</i>	
(ii) Distilled water	200ml



Procedure	
Take unfiltered sea water	2 litres
<i>plus</i>	
Soil extract	100ml
<i>plus</i>	
Nitrate/phosphate solution	2 ml
<i>plus</i> <b>(for diatoms only)</b>	
Silicate solution	2 ml
Decant <i>into</i>	250ml
8 clean flasks with cotton-wool plugs	500 ml (each)
Autoclave	35 min (as above)
Stand	for 2 days

**Table 3(b). The preparation of a simple medium**

Procedure	Quantities
Put sea water into glass or plastic container	2 l
<i>plus</i>	
<b>Solution A</b> (see Table 2)	3 ml
<i>plus</i>	
<b>Solution C</b> (see Table 2)	0.3 ml
<i>plus</i> <b>(for diatoms only)</b>	
<b>Solution D</b> (see Table 2)	3 ml
Mix	—
Into each of 8 flasks	250ml
<i>either</i>	
Heat	To boiling
<i>or</i>	
Autoclave	35 min at 1.06 kg per square cm (15 psi)
Cool*	Overnight

\*Note: Some precipitation may occur, but it will gradually re-dissolve.

## 4. SOME TYPES OF CULTURE

There are many different ways of culturing algae. These range from closely-controlled methods on the laboratory bench top, with a few litres of algae, to less predictable methods in outdoor tanks, containing thousands of litres, in which production relies on natural conditions. Several methods have been developed at Conwy, for the production of algae for use as food for various marine animals, and some of these methods are described in Section 5.

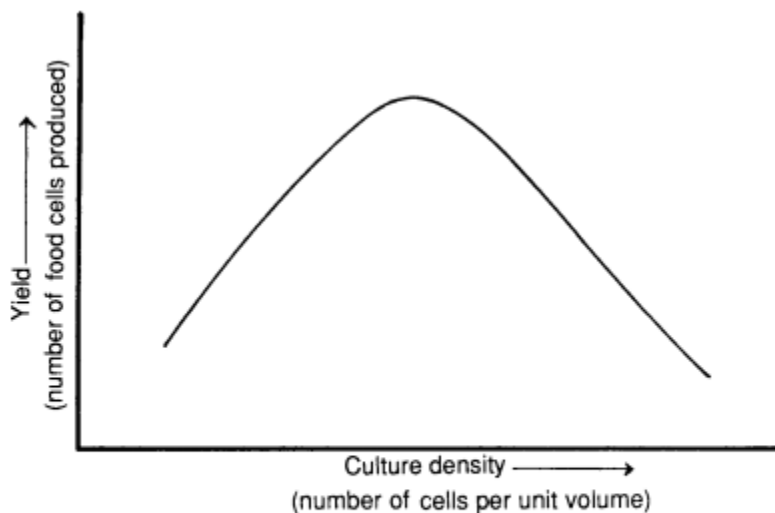
There are certain requirements for all methods. A culture must be inoculated, and the algae left to grow and divide. The rate of growth and division varies with different types of algae and also depends on how well the various culture conditions necessary for growth have been met. When there are sufficient algal cells in the container for feeding, one of the three culture methods below may be followed:

### 4.1 Batch culture

Batch culture is a system where the total culture is harvested and used as food. If required, another culture can be set up to replace it.

### 4.2 Semi-continuous culture

Semi-continuous culture is a system where part of the culture is harvested and used as food, and the amount taken is replaced with fresh culture medium (clean sea water and nutrient salts). After allowing 2-3 days for the remaining cells to grow and divide, the process is repeated. Semi-continuous cultures may be operated for up to 7 to 8 weeks.



**Figure 2.** *Variation in yield with the density of the algae culture*

### 4.3 Continuous culture

This falls into two categories:

- (i) *turbidostat culture*, in which the number of algal cells in the culture is monitored and, as the cells divide and grow, an automatic system keeps the culture density at a pre-set level by diluting the culture with fresh medium; and
- (ii) *chemostat culture*, in which a flow of fresh medium is introduced into the culture at a steady, pre-determined rate.

With both types, the surplus culture overflows into a collecting container, from which it can be taken and used as food.

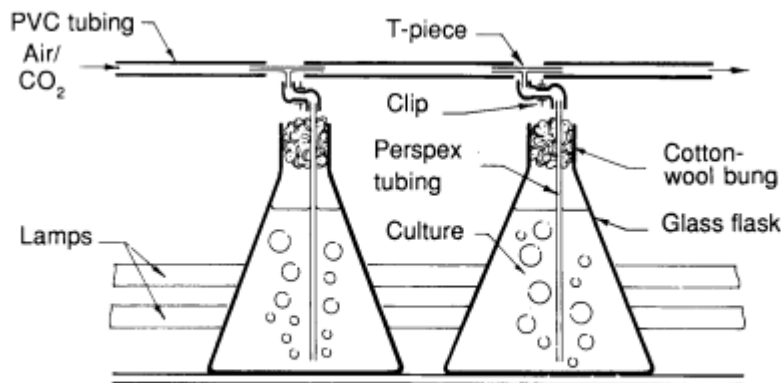
With semi-continuous and continuous culture methods, the number of food cells produced (the yield) varies with the density of the culture as shown in Figure 2. For each type of algae, the greatest yield is obtained by maintaining the culture at an optimum density. This optimum density can be determined experimentally and is given for each of the culture systems described in the following section.

## 5. SOME CULTURE METHODS

### 5.1 Batch culture (small volumes of up to 10 litres)

Where small volumes of algae culture are required, for example, of 2 litres to 10 litres per day, production is most conveniently achieved in flasks (Figure 3). An example of a batch culture system for producing 3 litres of food per day from a culture of *Chaetoceros calcitrans* is given below and is described as daily procedures in Table 4.

A set of three, 250 ml stock cultures is started by inoculating from one existing 250 ml stock culture on each of 3 successive days. The new stock cultures are grown at a temperature of about 21°C, and at a distance of 15 to 20 cm from a 65 watt fluorescent tube. After 3 days, and then daily, each of these



**Figure 3. Flask culture**

**Table 4. Schedule for a batch culture system, producing *Chaetoceros calcitrans* at a rate of 3 litres per day**

Day	Procedure	Inoculum
1	Inoculate new (250 ml) stock culture from existing culture  Grow in light	20ml
2	As Day 1	20ml
3	As Day 1	20ml
4	Inoculate new (250 ml) stock culture from Day 1 culture  Remainder starts (3 litre) batch culture  Grow in light	20ml  250ml
5	As Day 4, with Day 2 culture	20 ml+250 ml
6	As Day 4, with Day 3 culture	20 ml+250 ml
7	As Day 4, with Day 4 culture	20 ml+250 ml
8	As Day 4, with Day 5 culture. Use 3 litre batch culture started on Day 4 for feeding	20 ml+250 ml
9 (and onwards)	As Day 4, with stock culture started 3 days previously. Use 3 litre batch culture started 4 days previously	20ml+250 ml

stock cultures is used in turn to inoculate a new 250 ml stock culture and the remainder is added to the 3 litre sea-water culture medium in the flasks, which have been prepared as follows:

Three-litre borosilicate glass flasks with cotton wool plugs are filled with sea water. The contents are either autoclaved at 1.06 kg per square centimetre (15 psi) for 20 minutes, boiled for 30-45 minutes, or pasteurised. Whichever method is used, the sea water in the flasks should be allowed to cool before adding nutrient salts. Alternatively, sea water that has been filtered through a half micron filter may be used. To the 3 litres of sea water in the flask, 6 ml of solution A, 0.6 ml of solution C and 6 ml of solution D (see Table 2) are added.

A fresh 3 litre culture is started daily from a 3-day-old stock culture and aerated with a mixture of air and CO<sub>2</sub> at about 2 to 3 litres per minute. The gas mixture is filtered through an in-line cartridge unit containing a 0.3-0.45 micron filter to reduce the risk of airborne contamination. When grown at about 21°C next to a continually lit, double fluorescent lamp unit, a density of 45 000 to 60 000 cells per microlitre is reached in 3 to 4 days in culture medium prepared from heat-treated sea water. In medium prepared from filtered sea water, growth of *Chaetoceros* is not as rapid and the density will only reach 20 000 to 30 000 cells per microlitre in this time. The culture should immediately be used for feeding, as if kept it will enter a declining phase, collapse, and become unsuitable.

Algae other than *Chaetoceros* usually take longer to grow under similar culture conditions. For these algae, stock cultures should be routinely sub-cultured every 6 to 7 days. The batch cultures, which are started from 6- or 7-day-old stock cultures, will take 7 to 8 days to grow to a density suitable for feeding. Larger containers, of up to 10 litres, may be used for this method of culture. Nutrient salt solutions (see Table 2) are used as follows:

solution A — 1 ml per litre;  
solution C — 0.1 ml per litre; and  
solution D (diatoms only) — 2 ml per litre.

## 5.2 Semi-continuous culture

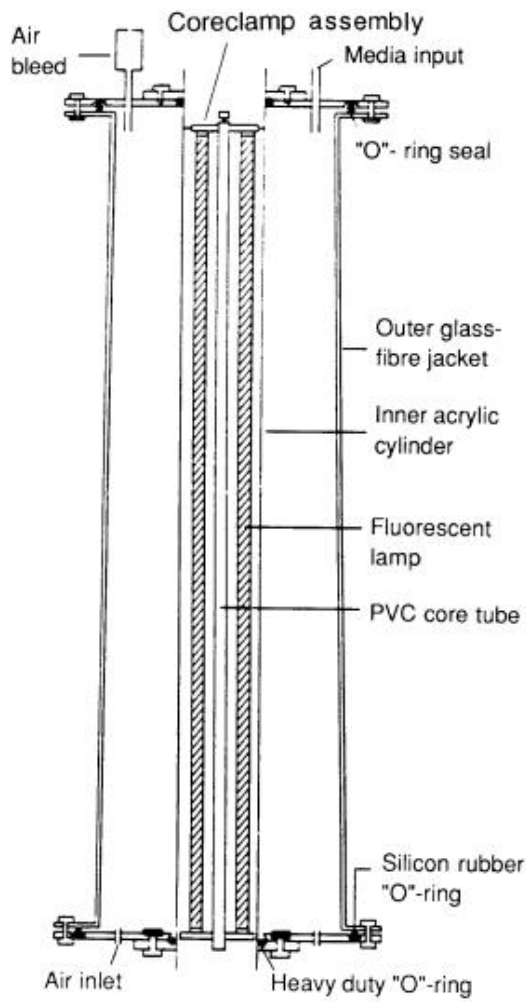
### 5.2.1 Two-hundred-litre vessels

A method for large-scale production (mass culture) in 200 litre, internally illuminated, glass reinforced plastic (grp) vessels (Figure 4), using semi-continuous culture is described below.

The vessels are 150 cm high, 40-45 cm in diameter and each has a central lighting unit into which are fitted six fluorescent lamps. A glass-fibre cooling pipe is moulded onto the outer jacket (Appendix 2). These vessels are most useful for growing diatoms, but they may also be used for flagellates. The vessels are sterilised by filling with a solution of sodium hypochlorite (50 parts per million (ppm) free-chlorine concentration). Note that domestic bleach contains about 100 000 to 150 000 ppm chlorine, so a dilution of 1 ml per 2-3 litres would give the required concentration. They are allowed to stand for 2-4 hours and then drained, and flushed with filtered air for 24 hours to drive off residual chlorine.

The vessels are filled (200 litres come to about 15 cm from the top) with filtered sea water at 20 psu to 25 psu salinity for diatom cultures or 25 psu to 30 psu for flagellate cultures. For diatom cultures, filtration to 2 microns is usually sufficient, while for culture of flagellates, filtration to half a

(a)



**Figure 4. Two-hundred litre, internally-illuminated, glass-reinforced plastic culture vessels: (a) longitudinal section; and (b) photograph of the system (for dimensions, see text)**

micron is preferable. Two-hundred millilitres of solution A, 20 ml of solution C and, for diatom cultures, 1 200 ml of solution D (see Table 2) are added to the vessels. The culture is inoculated with 2 to 5 litres of a 4- to 8-day-old batch culture, grown as described in the previous section and aerated with a filtered air/CO<sub>2</sub> supply at about 15 litres per minute.

Cultures should reach densities suitable for harvesting after 4 to 7 days. Those suitable for various types of algae are given in Table 5, which also gives the densities to which the cultures should subsequently be diluted for the maximum yield. The amount of harvest which achieves this yield can be calculated from the following equation:

$$\text{volume to harvest in litres} = 200 - \frac{200 \times \text{density to which culture needs to be diluted}}{\text{actual culture density of algae harvested}}$$

After harvesting, the vessels are topped-up to 200 litres with filtered sea water of the correct salinity. For each litre harvested, 1 ml of solution A, 0.1 ml of solution C and, for diatom cultures, 6 ml of solution D (see Table 2) are added. It is usually more convenient to harvest the culture every 2 to 3 days (e.g. Mondays, Wednesdays and Fridays). That part of the harvest which is required for feeding on the intermediate days can be aerated in a plastic container away from bright light and in a cool place.

**Table 5. Semi-continuous culture methods for various types of algae in 200 litre, internally-illuminated vessels**

Algae	Culture density (cells per microlitre)			Usual life of culture (weeks)
	Suitable for feeding	To which culture should be diluted for next harvest		
		in 2 days	in 3 days	
<b>Diatoms</b>				
<i>Skeletonema costatum</i>	6 000	1 250	625	6-7
<i>Thalassiosira pseudonana</i>				
<i>Phaeodactylum tricornerutum</i>	10 000	1 400	700	7-8
<i>Chaetoceros calcitrans</i>	15 000-20 000	4 800	2 400	2-3
<b>Flagellates</b>				
<i>Tetraselmis suecica</i>	1 000	160	80	7-8
<i>Chroomonas salina</i>	2 000	240	120	2-3
<i>Dunaliella tertiolecta</i>	3 000	350	175	4-5
<i>Isochrysis galbana</i>				
<i>Monochrysis lutheri</i>	10 000	1 440	720	2-3
<i>Pseudoisochrysis paradoxa</i>				

The length of time during which the culture is able to produce food will vary with the type of algae, as shown in Table 5. Production of algae from 200 litre vessels should average the equivalent of 60-80 litres per day at the cell densities given in Table 5. When the culture is no longer required, or it has come to the end of its production period, the vessel can be drained and cleaned with a stiff brush, to remove any algae adhering to the sides. The vessel can now be sterilised, as described above, in preparation for a new culture.

### 5.2.2 Sixty-litre polyethylene bags

A further large-scale production method utilises 25 cm wide polyethylene tubing made into bags and hung from a framework (Figure 5; see also Appendix 2). In the figure, the bag is shown folded in half, but it could also be cut to form two 30 litre bags, with the bottom of each heat-sealed across the end. This simple method for producing either diatoms or flagellates is suitable for use indoors with fluorescent lamps, and outdoors with natural daylight. The heat used in the manufacture of the bags ensures that they are sterile when supplied.

When setting up a system, a small hole is made in the top of each half of the bag and filtered sea water, at 20 psu to 25 psu salinity for diatom culture and 25 psu to 30 psu for flagellates, is pumped-in until the bag is almost filled. To each half, 30 ml of solution A, 3 ml of solution C and (for diatoms) 150 ml of solution D are added (see Table 2), and then about 2 litres of a batch culture that has grown for 4-8 days. An air line of 7 mm diameter Perspex tubing is fitted into each half of the bag and aerated vigorously. The culture is allowed to grow.

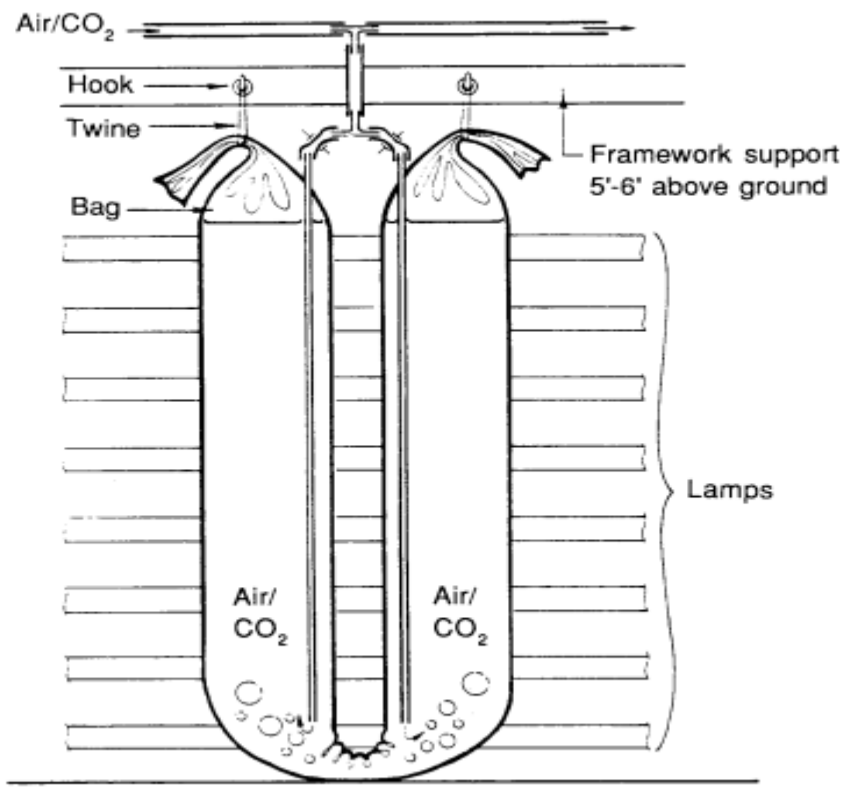


Figure 5. Sixty-litre polyethylene bag culture



From an indoor bag system, continually illuminated diatom cultures may be harvested after 2-3 days, and flagellates after 4-5 days. Harvests can then be taken three times per week (e.g. on Mondays, Wednesdays and Fridays) by either pumping or siphoning-out the algae cultures, removing 50 litres from diatom cultures and 30 litres from flagellate cultures. (For siphoning, the framework must be raised or a sunken area provided to give a sufficient head of water.) That part of the harvested culture required for feeding on the intermediate days (Tuesdays, Thursdays, Saturdays and Sundays) can be aerated in a plastic container away from bright light.

The culture in the bag is topped-up with filtered sea water at the correct salinity, added to both sides of the bag to ensure that the culture remains evenly mixed. For flagellates, 15 ml of solution A and 1.5 ml of solution C or (for diatoms) 25 ml of solution A, 2.5 ml of solution C and 125 ml of solution D, should be added to each side of the bag (see Table 2). After harvesting for three weeks, production from the culture becomes unreliable, so it is wise to use all of the culture for feeding and discard the bag. A fresh culture can now be started in a new bag.

To ensure a continual supply of algae, one-third of the required number of culture bags should be started at a time in rotation (i.e. for every three bags of a particular species of algae, start one the first week, another the second week and the third the following week).

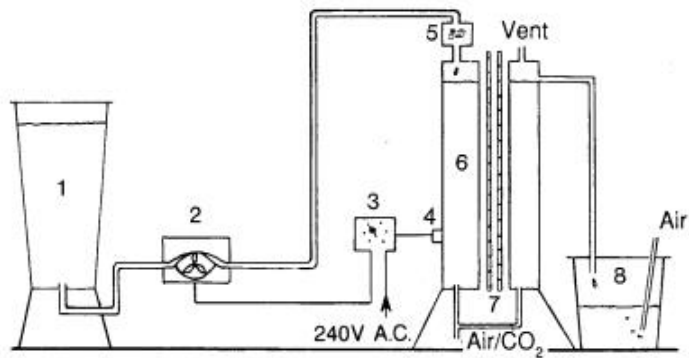
Production in outdoor bag cultures is very variable, depending upon the amount of light available and also on the temperature. Direct sunlight should be avoided, as this causes the cultures to become too hot and to collapse. The framework for the bags should therefore face north or (if impracticable) suitable shading should be provided. As the rate of growth will be variable, an estimate of culture density, using an haemocytometer, will be required (see Appendix 1). Cell densities suitable for harvesting and feeding are given in the first column of Table 5.

When these densities have been reached or exceeded, the above procedures should be followed for harvesting and topping-up the bags with fresh medium. After about ten semi-continuous harvests, the culture and bag should be replaced.

### **5.3 Continuous culture (40 litre vessels)**

This method is suitable for the culture of flagellates. The internally-illuminated, continuous culture vessels are made from polyethylene tubing supported by a metal framework (Figures 7 and 8; see also Appendix 2). They consist of 160 cm lengths cut from 71 cm wide polyethylene, 'layflat' tubing. The tubing is free of potential contaminants due to the heat used in the manufacturing process and no further sterilisation is necessary. The cut length is heat-sealed across the width of one end and positioned around the acrylic cylinder containing the lamps. The six nuts and bolts securing the outer supporting mesh jacket are fastened and the outer, reflective, sheet of white, corrugated plastic is held in place by 12.7 mm nylon power belting (nylon strapping), which also supports the sensor housing unit against the outer surface of the culture.

The polyethylene tubing is filled (38 litres) with sea water at 25 psu to 30 psu salinity that has been filtered through a sterile, 0.45 micron, particle retention cartridge filter. If the water has a high silt load, it should first be passed through a 2 micron filter. Solution A (100 ml) and solution C (10 ml) (see Table 2) are added to the sea water in the vessel. This is 2.5 times the usual amount, and is added to ensure that nutrient levels do not become limiting at the high cell



**Figure 7. Diagram of continuous culture apparatus (not drawn to scale): (1) sea-water medium reservoir (200 litres); (2) peristaltic pump; (3) resistance sensing relay (50-5 000 ohm); (4) light-dependent resistor (ORP 12); (5) cartridge filter (0.45 micron); (6) culture vessel (40 litres); (7) six 80 W fluorescent lamps; and (8) collecting reservoir (125 litres)**

densities at which the cultures are maintained. A 2.5 cm diameter circle is cut from the tubing, with its centre about 7 cm above the water level. Into this is fitted a 1.9 cm rigid, PVC tank connector, from which a 150 cm length of 1.5 cm bore flexible PVC tubing is run into a 125 litre collecting vessel. The overflow allows for automatic harvesting of the culture into the reservoir.

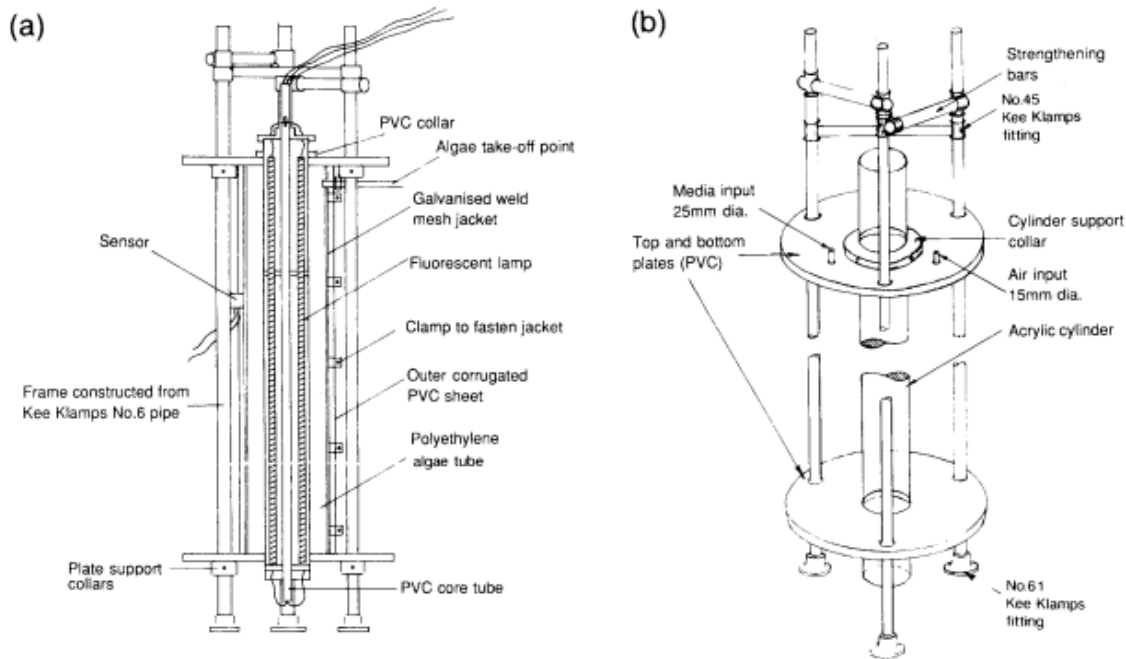
A supply of filtered air, enriched with sufficient carbon dioxide to maintain culture pH at 7.6-7.8 (about 0.25% CO<sub>2</sub> by volume) is introduced through a 0.4 cm bore, 150 cm long, acrylic tube inserted into the top of the culture. A flow rate of about 15 litres per minute ensures efficient mixing of the culture.

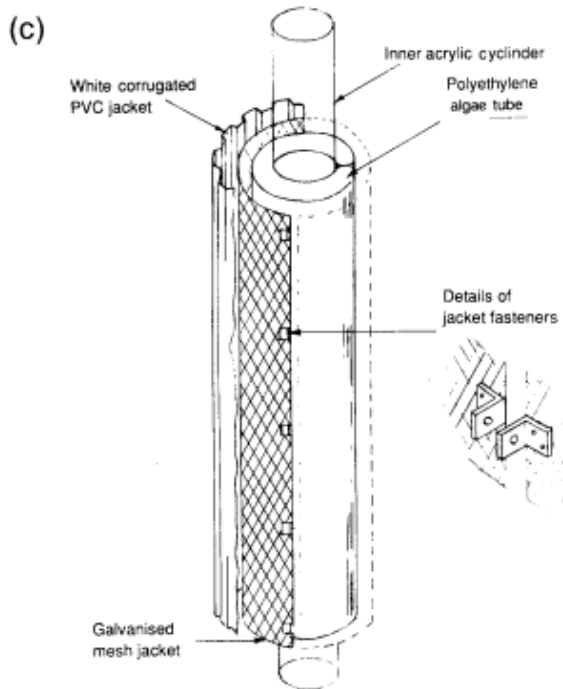
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Cooling water, at a flow rate of about 0.35 litre per minute, is allowed to run down over the outer culture surface in order to maintain the culture temperature at about 21°C.

The 40 litre culture should be inoculated with a 2 litre batch culture that has grown for 7 to 8 days.

Automatic harvesting of the culture is controlled by the following method. A cadmium sulphide photo-conductive cell is enclosed in a light-proof housing against the outer surface of the culture. The housing is placed about 50 cm from the base of the vessel and positioned so that the stream of air bubbles rising through the culture does not interfere with its operation. The resistance of the photo-conductive cell will increase as the light intensity reaching it from the lamps falls, when density of the culture increases, due to growth and division of the algal cells. A circuit switching the peristaltic pump will be energised when the resistance of the cell becomes greater than a present value on a relay sensitive to input resistance in the range 50-5 000 ohm. The 11-pin relay, connected to the light-dependent resistor and peristaltic pump, is shown in Figure 9. Sea water, at 25 psu to 30 psu and enriched with 2.5 ml of solution A per litre and 0.25 ml of solution C per litre, is then pumped from the culture medium reservoir through the filter into the vessel and the volume is maintained by an overflow. The outflow of algae culture from the vessel is collected in an aerated container. As the culture is diluted, the decrease in resistance of the photo-conductive cell, caused by the higher light intensity now reaching it, is sensed by the relay and the pump circuit is switched off.





**Figure 8. Construction of a 40 litre continuous culture vessel: (a) general arrangement; (b) supporting frame; and (c) arrangement of the algae culture tube**

The relay should be set so that automatic harvesting of the culture occurs at the density that gives the most yield. The optimum densities for some types of commonly grown algae are given in Table 6, together with the expected lives of the cultures. Production of 30-40 litres per day, at the cell densities given in Table 6, can be expected from these 40 litre vessels. When the yield begins to fall appreciably, all of the culture should be harvested for feeding and the bag discarded. A new, clean bag should be fitted to the vessel and the above operating procedure repeated.

## Indian contribution to Algology

- (1) Study of algae is known as phycology or algology.
- (2) The term 'Phycology' is derived from two greek words Phykos, greek name of an alga + logos, study or discourse.
- (3) The term 'algae' was given by- Linnaeus (derive from a Latin word which means sea weads.)
- (4) Algae include the chlorophyllous non-vascular plants with thallose plant body i.e. with no differentiation of root, stem and leaves.
- (5) Dr. F.E. Fritsch famous international algologists and he is known as Father of Algae.
- (6) Prof. M.O.P. Iyenger he was student of Dr.F.E. Fritsch, started study of algae in 1920 in Madras University.

He is known as Father of Modern algology in India. His important contribution is discovered of terrestrial alga called *Fitschiella tuberosa*.

- (7) In BHU Prof. Y. Bhardwaj (student of Fritsch) and Prof. R.N. Singh his important work is reclamation of saline user land by growing blue green algae also role of blue green algae in nitrogen economy of Indian agriculture. (although P.K. De was the pioneer in establishing the N<sub>2</sub>-fixing ability of blue green algae.)
- (8) Prof. H.D. Kumar worked on algae Physiology. He also studied genetic recombination in BGA or Cyanophyceae or Myxophyceae or cyanobacteria.
- (9) Dr. F.E. Fritsch (1935) regarded the class as a highest grade in algae (phyceae) and on the basis of pigmentation, product of assimilation and ciliation divided Algae into following 11 classes
- (i) Chlorophyceae
  - (ii) Xanthophyceae
  - (iii) Chrysophyceae
  - (iv) Bacillariophyceae
  - (v) Cryptophyceae
  - (vi) Dinophyceae
  - (vii) Chloromonadinaceae
  - (viii) Euglenophyceae
  - (ix) Phaeophyceae
  - (x) Rhodophyceae
  - (xi) Myxophyceae or Cyanophyceae
- (10) Algae occur in a variety of habitats, but majority of them are aquatic. They show distinct alternation of generation.

### **Economic Importance of Algae**

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1. Algae Constitute the Link of Food Chain
2. Algae is Useful in Fish Culture
3. Algae is Used for Recreational Purposes
4. Algae is Useful in Sewage Treatment Plants
5. Algae and Water Supplies
6. Algae as the Origin of Petroleum and Gas
7. Algae and Limestone Formation
8. Algae is Used in Space Research and Other Fundamental Studies

9. Algae is Used as Food
  10. Algae is Used as Fodder
  11. Algae is Used as Fertilizers
  12. Algae is Used as Medicine
  13. Industrial Utilization of Algae
- 

### **1. Algae Constitute the Link of Food Chain:**

Both fresh and salt waters contain an enormous variety of algae which constitute the fundamental or primary link of many diverse food chains. Algae synthesize organic food stuffs, just as do the plants of the land. As the flesh of the land is dependent upon the activities of the green leaf, so the fish and other aquatic forms of animal life are dependent, directly or indirectly, upon algae, and fish in turn are important item in the daily diet of larger sea animals and man.

A number of aquatic algae form the food of fish either directly or indirectly. Diatoms, filamentous and some planktonic green algae, and a number of blue-green algae are very often found in the guts of various species of fresh and brackish water fish and they appear to be directly utilized as fish food.

The reserve food materials in these algae, e.g., fats and volutin in the diatoms; starch, often accompanied by oil in the green algae; sugars and glycogen in the blue-green algae; and polysaccharides in Euglena are utilized by fish.

### **2. Algae is Useful in Fish Culture:**

That algae are fruitfully utilized in fish culture can very well be indicated from the successful culture of the Siamese fish, *Tilapia mossambica* which is voracious feeder of filamentous algae. This particular fish has been successfully introduced in

different parts of India. A culture of *Scenedesmus* is often exclusively used as a daily dose of fish meal for the culture *T. mossambica*.

### **3. Algae is Used for Recreational Purposes:**

Certain selected algae are grown in recreational areas like—lakes and streams along with fish.

### **4. Algae is Useful in Sewage Treatment Plants:**

Species of *Chlamydomonas*, *Scenedesmus*, *Chlorella* and *Euglena* are used in sewage treatment plants for providing through photosynthesis the oxygen necessary for rapid decomposition of the sewage by bacteria.

### **5. Algae and Water Supplies:**

In the summer months the phytoplankton in ponds, lakes and reservoirs may become so abundant as to be extremely conspicuous. The water becomes cloudy and may assume a yellowish or greenish tinge. A floating mat of scum may develop.

These manifestations of algal growth are popularly termed ‘water bloom’. Such concentrations of algae are extremely objectionable, not only in public water supplies but also in waters used for bathing, fishing and other recreational purposes.

The blue-green algae are most frequently involved in the contamination of water supplies, but the greens, the flagellated golden-brown, and the diatoms are also troublesome at times. Mention may be made of *Prymnesium parvum*, *Gymnodinium veneficum* and *Microcystis* spp. which cause mortality of fish and domestic animals that drink water infested with these algae.

The living and the dead and decaying algae impart disagreeable oily or fishy odours to the water.

Toxic protein decomposition products may be formed by blue-green algae and these are known to have caused death of cattle, sheep, and other animals which have drunk heavily infested water. In addition to the unsightly appearance and the unpleasant odours and tastes, the presence of algae in reservoirs requires a greater concentration of chlorine for bacterial control and causes difficulties in filtration.

## **6. Algae as the Origin of Petroleum and Gas:**

The origin of oil and gas has been a matter of controversy, but it is now generally believed that, like coal, these fuels owe their energy to photosynthesis in ancient plants. Unlike coal, however, which was laid down in inland swamps, oil and gas were formed from organic matter in marine environments.

The plankton of the seas was probably of the greatest importance as a source of this organic matter. Minute marine algae captured the energy of sunlight, which was in turn transferred to the animals that fed upon them.

Organic compounds derived from the plankton, both plant and animal, accumulated in mud deposits in shallow waters of the ocean floor. In the source, materials were buried by sedimentary action and, in an oxygen-free environment, gradually converted into oil and gas.

Natural gas is largely methane ( $\text{CH}_4$ ), which can be produced by certain kinds of anaerobic bacteria. Gas is generally associated with oil and can result from the action of methane-producing bacteria upon organic compounds.

## **7. Algae and Limestone Formation:**



Many species of algae withdraw calcium from water, both fresh and salt, and deposit it, in the form of calcium carbonate, in their cell walls or gelatinous sheaths. The most significant forms in this category are the blue-greens and reds, but certain green algae and flagellates are also concerned.

The blue-greens are chiefly important in fresh-waters; they are responsible, for example, for the formation of extensive limestone deposits around hot springs and glaciers. The red algae are the most important calcareous algae of the seas; in particular, they play a significant role in the construction of coral reefs and islands.

Although true coral results from the activities of minute sedentary animals, it is recognized that lime-secreting red algae are almost as important in the formation of coral reefs as the coral organisms themselves. The calcareous red algae are best developed in the warmer seas, but certain species also flourish in temperate and polar regions, where they form extensive banks of limestone in coastal areas.

The algae are not only important in the present age in the formation of calcareous deposits, both in the seas and fresh waters, but also they have played a significant part in the production of beds of limestone rocks, which may be 1000 feet thick.

### **8. Algae is Used in Space Research and Other Fundamental Studies:**

In recent years Chlorella is being used in space research. Chlorella has been found very suitable for keeping the air in space vehicles pure on long interplanetary flights. The stale air in which the carbon dioxide has been concentrated is fed into a flood-lit container containing a mixture of water and nutrient chemicals and Chlorella.

The alga restores oxygen into the space vehicle by its photosynthesis. Again species of *Chlorella*, *Chlamydomonas*, and *Acetabularia* are used as tools for solving fundamental biochemical and genetical problems.

### **9. Algae is Used as Food:**

Large number of algae have entered into the diets of human beings from ancient times. The earliest records are those of the Chinese, who mentioned such food plants as *Laminaria* and *Gracilaria* in their 'materia medica' several thousand years ago.

The ancient inhabitants of Japan ate *Porphyra* as a healthful supplement to their rice diet. Its use became widespread, not only in Japan, but in China in course of time. *Kombu*, a Japanese food is prepared from stipes of species of *Laminaria*.

The most diversified dietary use of seaweeds was developed by the Polynesians and reached its peak in Hawaii, where during the nineteenth century at least 75 species were separately named and used regularly as food in that island world. The Hawaiians called them 'limu' and considered them a necessary staple of their daily diet.

Perhaps the best known and most widely used food alga in Western Europe in recent centuries was Irish moss, or carrageen (*Chondrus crispus*), which was cooked with milk, seasoned with vanilla or fruit, and made into a highly palatable dish known, as *blancmanges*. The jellying qualities of Irish moss gave the alga an early food use.

Man, thus obtains carbohydrates, vitamins (algae are especially rich in vitamins A and E, and they contain some C and D), and inorganic substances, e.g., iodine (goiter is unknown among the people who eat seaweeds), not to mention the benefits of the

mild laxative action of the ingested algae. Witsch (1959) stated that vitamin B value of young cultures of *Chlorella* equals that of lemon juice.

In Japan, powdered *Chlorella ellipsoidea* has been used successfully mixing with green tea.

In Germany and in the United States considerable work is being carried out on the suitability of mass cultures of *Chlorella* as an alternative source of animal feed and of human vegetable food.

#### **10. Algae is Used as Fodder:**

The orientals developed wide human uses for marine algae, but Europeans profited by extensive use of these plants for stock feed. In Iceland and Scandinavia, in the British Isles, and along the coast of France, stock has long been driven or allowed to wander to the seashore at low tide to feed on seaweeds.

Some kinds of algae, such as *Rhodomenia palmata* and *Alaria esculenta*, are favourable food of goats, cows, and sheep, and in Scotland and Ireland the stock actively hunt the shores at low tide for particular algae, especially the former.

The milk does not have any taste of algae, nor is the meat inferior because of the seaweed diet. Such animals, which have for several generations been nourished on algae, show better ability to digest it than those not so habituated.

The shortage of grain in many parts of Europe during World War I led to considerable experimentation with the use of seaweeds as food for cows and horses. Stock-feed factories were established in France, Norway, Denmark and Germany,

and various methods of treating and reducing seaweeds to meal or powder were developed.

The favourable results in animal husbandry in Europe led to the industrial processing of the great Pacific-Coast kelp (*Macrocystis*) for animal rations. Seaweed-meal factories have been operating in the United States for several decades, providing supplementary feeds for poultry, cattle and hogs.

The high mineral and vitamin G content of kelp meal has made possible its use in various poultry and other animal rations.

### **11. Algae is Used as Fertilizers:**

The value of seaweeds in fertilizing the soil was discovered early in the history of agriculture in coastal Asia, and by the ancient colonizers of the coasts and islands of North-Western Europe. In some areas of Britain, and along the coast of North-West France, the cutting of rockweeds for manure has been so intensively practiced that it became necessary to regulate it by laws that have now been in effect for nearly 100 years.

In the United States, long before the recognition of their potash content, seaweeds were employed for fertilizers by the thrifty farmers. Not only the chemical fertilization, but also the water-holding capacity of fragments of the algae in the soil proved effective. These provided valuable small reservoirs of water in close contact with the roots of the cultivated plants.

Various blue green algae such as *Oscillatoria*, *Anabaena*, *Nostoc*, *Aulosira* increase the soil fertility by fixing the atmospheric nitrogen. In view of the increasing energy demands and rising costs of chemically making nitrogenous fertilizers, much

attention is now being given to nitrogen fixing bacteria and blue green algae. Many species of sea weeds are used as fertilizers in China and Japan.

Algae are a large and diverse group of microorganisms that can carry out photosynthesis since they capture energy from sunlight. Algae play an important role in agriculture where they are used as biofertilizer and soil stabilizers. Algae, particularly the seaweeds, are used as fertilizers, resulting in less nitrogen and phosphorous runoff than the one from the use of livestock manure. This in turn, increases the quality of water flowing into rivers and oceans. These organisms are cultivated around the world and used as human food supplements. They can produce a clean and carbon-neutral food also and can be grown on abandoned lands and arid desert lands with minimal demands for fresh water. Seaweeds are an important source of iodine. Iodine levels in milk depend on what the cow producing the milk has been fed with. Feeding milk cattle with seaweeds can increase the quantity of iodine in milk, according to Fuzhou Wonderful Biological Technology. Egg-laying rate in hen is also increased by algae feed additives.

**IMPROVEMENT OF SOIL FERTILITY** Some cyanobacteria are able to reduce atmospheric nitrogen to ammonia, a process where oxygen evolved by photosynthetic activity in the same cell is detrimental to nitrogen fixation. Strategies to avoid oxygen range from temporal separation of nitrogen fixation and oxygen evolution (in many unicellular and filamentous, non heterocysts strains) to spatial separation and cellular differentiation into nitrogen fixing heterocysts (in filamentous cyanobacteria). Heterocysts are terminally differentiated cells whose interior becomes anaerobic, mainly as a consequence of respiration, allowing the oxygen-sensitive process of nitrogen fixation to continue.

**Nitrogen fixation Algae**, especially cyanobacteria, may be the most important nitrogen-fixing agents in many agricultural soils. Cyanobacteria are widely used in rice fields throughout Asia, where their enhancement of soil fertility by means of biological nitrogen fixation (so called algalization) in place of N-rich fertilizers.

**Source of organic matter**, Algae are also important source of organic matter in soil. The organic matter formed from the death and decay of algae may get mixed in the soil and mucilage acts as binding agent for soil texture, thereby increasing the humus content and making it more habitable for other plants after some years. The filamentous forms of the Cyanophyceae, especially *Oscillatoria*, *Schizothrix* and *Plectonema* were found to be important in soil formation (Gayel and Shtina, 1974). In most cases, it is generally accepted that the incorporation of organic carbon via photosynthesis and of organic nitrogen via nitrogen fixation is the most important contributions of algae added to the soil. They also act as a reserve of inorganic nutrients.

**Soil reclamation** The difficulties in soil reclamation in arid and semi-arid regions are mostly the salinity conditions of large soil areas. Some growth regulators such gibberellic acid (GA3) were used for improving the salt tolerance of the plants (Ouda et al., 1991). From an economic point of view, growth regulators are expensive and are non-practical especially, when applied in large amounts. Algae play an economic role in soil reclamation increases soil fertility and improve the plant conditions under certain environmental factors.

Blue-green algae, especially the nitrogenfixers cyanobacteria, represent the major microorganisms which contribute soil fertility. These organisms play an important role in this system by providing a steady input of fixed nitrogen.

Furthermore, the bulky organic substances decay slowly in the soil and form humus. Again yield of paddy is increased substantially when paddy field is inoculated with nitrogen fixing blue-green algae. Some of them are: *Tolypothrix tenius*, *Aulosira fertilissima*, *Anabaena oryzae*, *Anabaenopsis arnoldii*, *Calothrix confervicola*, *Nostoc commune*, and *Cylindrospermum bengalense*.

## **12. Algae is Used as Medicine:**

Medicinal applications of plants are almost as old as their food uses. From earliest times the Chinese used *Sargassum* and various *Laminaria*-riales for treatment of goiter and other glandular troubles. *Gelidium* very early became employed for stomach disorders and for heat-induced illness.

The gentle swelling of dried *Laminaria* stipes upon exposure to moisture make them surgical tool in the opening of wounds. Similarly, the orientals have employed the same technique in child-birth for expansion of the cervix.

Perhaps the algae used most widely and for the longest time for medicinal purposes and from which agar is extracted are the agarphytes, including *Gelidium*, *Pterocladia*, *Gracilaria*, and *Ahnfeltia*. The name 'agar-agar' is of Malay origin and means 'jelly'. This jelly was obtained by boiling up seaweeds and cooling the resulting liquid.

Agar early became useful for stomach disorders and as a laxative, and was once employed as a dietetic. It was originally produced and marketed in China, but the

Japanese took over production in about 1662 and maintained a world monopoly till 1940.

The most significant date in the utilization of agarphytes was 1881, when Robert Koch proved the value of agar in the cultivation of bacteria. Since that time it has become essential to the work of hospitals and medical research laboratories throughout the world. Besides these, *Chlorella* is used for the preparation of antibiotic Chlorellin.

### **13. Industrial Utilization of Algae:**

#### **(i) Kelp Industry:**

Industrial utilization of seaweeds in Europe had its principal early development in the production of 'kelp', a name that originally referred to the ash, rich in soda and potash, derived from burning marine plants. Kelp production was begun sometimes in the seventeenth century by French peasants and spread to other parts of North-West Europe.

Drift-weeds were first used, but cutting was later resorted to *Laminaria* and *Saccorhiza* in North Britain as of major importance.

But *Fucus* and *Ascophyllum* were also widely used, and in some areas *Himanthalia* and *Chorda*. The kelp ash from these plants was widely bought by early industrialists for use in manufacture of soap, glass and alum. During the eighteenth and early nineteenth centuries the demands became considerable, and enormous quantities of seaweeds were handled in areas of rich algal growth.

Kelp extract contains a number of chemical elements, notably potassium and iodine. About 25 per cent, of the dry weight of kelp is potassium chloride. Many species of



kelp are used as food for man, especially in the Orient. In Northern Europe they also serve as food for domestic animals, such as sheep and cattle.

**(ii) Algin Industry:**

Algin is the general term designating the hydrophilic, or water-loving derivatives of alginic acid. The most commonly known algin is sodium alginate, but other commercially important compounds are the potassium, ammonium, calcium, and propylene glycol alginates, as well as alginic acid itself.

With the exception of alginic acid and calcium, alginate, the algin products offered commercially are soluble in water to form vis-Sous solutions.

Algin occurs generally throughout the brown algae (*Laminaria*, *Macrocystis*, *Sargassum* and *Fucus*) as a cell wall constituent. It has remarkable water-absorbing qualities that make it useful in numerous industries in which a thickening, suspending, stabilizing, emulsifying, gel-forming, or film-forming colloid is required.

Thus, algin provides ice cream with a smooth texture by preventing the formation of ice crystals. In automobile polishes it suspends the abrasive; in paints, the pigments; also in pharmaceuticals, the drugs and antibiotics. As a stabilizing agent it serves in the processing of rubber latex .and in the printing of textiles. As an emulsifier it is widely used in such products as water-based paints, French dressings, and cosmetics.

The algin industry has become so important to such a wide variety of industries that extensive survey of kelp-bed ecology is an effort to guard against loss of this important resource. Harvesting methods are now carefully regulated, and a huge amount of money is being spent on kelp-bed research throughout the world.

Experimental studies are continuing on the relation of pollution to kelp survival and on kelp-bed grazing organisms.

### **(iii) Agar Industry:**

The outstanding use of the red algae, however, is in the production of agar. This is a dried and bleached gelatinous extract obtained from red algae—*Gelidium nudifrons*, *G. pusillum*, *G. robustum*, and *Gracilaria verrucosa*. Agar is used extensively in medicine, chiefly as laxative, since it is not digested and increases greatly in bulk with the absorption of water.

More important than this medicinal utilization is its use as an essential ingredient in the preparation of medium for the growth of bacteria and fungi. As such it is indispensable in bacteriological laboratories, because no adequate substitute for agar is known.

Since the introduction of agar into bacteriology in 1881, the agarphytes have become increasingly industrialized and the technical uses of agar enormously expanded. Modern industry has developed such a multitude of applications that only a fraction of them can be noted here. Large quantities of agar are used as a food adjunct.

Agar serves widely as a substitute for gelatin, as an anti-drying agent in breads and pastry, in improving the slicing quality of cheese, in the preparation of rapid-setting jellies and desserts, and in the manufacture of frozen dairy products. The use of agar in meat and fish canning has greatly expanded, and hundreds of tons are utilized annually.

Agar has proved effective as a temporary preventive for meat and fish in tropical regions, due to the inability of most purifying bacteria to attack it.

Early industrial uses of agar in the Orient included sizing fabric, water-proofing paper and cloth, and making rice paper more durable. Modern industry has refined and expanded these uses to meet new needs in the manufacturing of such items as photographic film, shoe polish, dental impression molds, shaving soaps, and hand lotions.

In the tanning industry agar imparts a gloss and stiffness to finished leather. In the manufacture of electric lamps, a lubricant of graphite and agar is used in drawing the hot tungsten wire.

The increasing applications have called for wide expansion of the collection of agarphytes, and since Japan supplied most of the world's markets before World War II, when those supplies were cut off, a great amount of hurried research was conducted in an attempt to develop domestic agar supplies not only in the United States, but in South Africa, Australia, New Zealand and Russia.

#### **(iv) Diatomaceous Earth Industry:**

The Diatoms are equally important in comparison with other algae that have industrial utilization. Most species of Diatoms are marine, and when these minute plants die, they fall to the sea bottom and, because of their siliceous nature, the cell walls are preserved indefinitely. Great deposits of this material, known as diatomaceous earth, are found in many parts of the world.

The largest beds in the United States, some 1400 feet thick, are in California. The beds are sedimentary deposits originally laid down on the floor of the ocean and later raised above the level of the sea.

Because diatomaceous earth is inert chemically and has unusual physical properties, it has become an important and valuable material in industry. It makes an excellent

filtering agent, which is widely used to remove colouring matters from products as diverse as petrol and sugar.

As a poor conductor of heat it is used in soundproofing. It is used in the manufacture of paints and varnishes, of phonograph records, and as a filler for battery boxes. Because of its hardness, it is used as an abrasive in scouring and polishing powders.

## **Plankton**

Plankton lack any type of self-propelled mobility. The current in the surrounding water propels them. This form of motion helps to disperse the organisms throughout a body of water. Plankton occupy the pelagic zone of the water column, which is named after its pelagic inhabitants.

Plankton may range in size from less than 2 micrometers to organisms larger than 200 micrometers. The category includes many different species of organisms in ocean and freshwater ecosystems. Plankton are divided into phytoplankton and zooplankton. Phytoplankton are photosynthetic and act as the primary producers in an aquatic environment. Zooplankton are heterotrophic and consume smaller plankton.

## **Phytoplankton**

Phytoplankton are the primary producers of their environment, meaning they are the first organisms to produce energy, which they create from light sources, such as the Sun. They convert acquired light energy into carbohydrates through photosynthesis. Energy not used by the phytoplankton for maintenance is available as food for the animals that consume it.

Phytoplankton absorb about 3 percent of the light shining on the ocean. By comparison, plants on land absorb about 15 percent of the available sunlight. This discrepancy is caused by the ocean itself, which absorbs sunlight in varying degrees. This competition for vital light resources is a limiting factor for the rate of primary production in aquatic ecosystems.

Phytoplankton are tiny photosynthetic organisms that are the major producers of marine life. They form the foundation of the food web for most marine life. They are responsible for half of the photosynthetic activity on earth, making them important to both their local and the global ecosystems. They are comprised of beings from different kingdoms. Their importance in carbon-dioxide sequestration has made them a target for controlling carbon dioxide in the atmosphere.

Phytoplankton are the photosynthetic portion of plankton life. Plankton are the tiny, drifting organisms that live in the top layers of ocean and lakes. Because phytoplankton rely on sunlight to produce their own food, they are found in the top layer of water. This layer, the epipelagic layer, goes down 200 meters. It is defined by the fact that enough light gets through the water to allow for photosynthesis.

### **Marine Ecosystems**

Phytoplankton are vital to marine ecosystems. They are producers, or autotrophs, that form the foundation of most marine food webs. As photosynthetic organisms, they are able to convert solar energy into chemical energy and store it as sugars. Consumers, or heterotrophs, must consume energy that has already been converted into chemical energy. Consumers can either eat autotrophs directly, or eat other consumers. Phytoplankton are eaten by other small organisms, such as zooplankton.

## **Global Ecosystems**

Phytoplankton are important to the global ecosystem as well as oceanic ecosystems. They are responsible for half of the photosynthetic activity on the planet. This means that of the carbon dioxide in the atmosphere that gets fixed into sugars, phytoplankton are doing half of the work. This makes them important to global carbon-dioxide levels. Without phytoplankton to pull carbon dioxide out of the atmosphere, carbon-dioxide levels would rise, because carbon dioxide would continue to be produced in both biological and industrial sources.

## **Types**

Phytoplankton are in a group because of the ecological role, or niche, that they play. They consist of plants, animals, archaea and bacteria. Three of the major types of phytoplankton include diatoms, dinoflagellates and microflagellates. Diatoms are relatively large, reaching .2 mm in length, divide rapidly, and have minimal ability to control their movement. Dinoflagellates are smaller, divide less rapidly and have flagella to regulate their position in the water. Microflagellates are very small, divide slowly and, like dinoflagellates, have flagella for maneuvering.

## **Ocean Health Depends on Phytoplankton**

The importance of phytoplankton is due largely to their place at the base of the marine food chain. Small fish and some larger species of fish and whales consume phytoplankton as their main food source. These fish then become prey for larger fish and marine mammals on up the chain. Dead phytoplankton fall to the bottom of the ocean and nourish shellfish and other bottom dwellers. Crashes in the phytoplankton population can have serious ramifications for the entire marine

ecosystem. Variances in the phytoplankton population can be an indicator of other ocean problems, such as excessive pollution.

### **Phytoplankton Influence the Global Climate**

Global climate health is affected by phytoplankton population health. Phytoplankton is responsible for approximately 50 percent of all photosynthesis on Earth. This means they function as a major carbon dioxide sink, pulling the gas from the atmosphere and emitting oxygen in its place. In this way the phytoplankton population is a major factor in limiting global warming and in the general atmospheric health of the planet.

### **A Factor in Human Health**

The importance of plankton doesn't stop in the water: the health of the human population is directly related to the health of the oceans and the climate. Certain species of fish that consume phytoplankton, such as sardines, serve as a food source both for humans and larger fish. Many communities worldwide depend on commercial fishing both for nourishment and employment. Without phytoplankton, the fish population and therefore commercial fishing would disappear. Humankind will also be impacted in many ways by global warming, and phytoplankton's key role in this process makes them critical to our survival.

### **Population Variance**

Concerns have been raised by scientists that the hole in the ozone layer could have negative impacts on the phytoplankton population, as harmful rays from the sun could kill them. Phytoplankton are also harmed by pollutants in the ocean, such as agricultural and industrial runoff, and are often absent where pollutant

concentrations are high. Nourished by nutrients welling up from the ocean floor and iron deposited on the ocean's surface by the wind, phytoplankton are at risk from changes in global climate and wind patterns. Winds drive the current upwellings which nourish the phytoplankton and also carry required minerals to the ocean. Dust from drier climate conditions can limit the sunlight and hurt the ability of phytoplankton to perform photosynthesis and survive.

### **Monitoring Phytoplankton**

Studies are being conducted by scientists worldwide in attempts to understand the phytoplankton populations and the factors affecting it. Tracking seawater's color change from blue to green as phytoplankton density increases has been done for decades. New technologies are allowing scientists to also determine the health and growth rates of the organisms using NASA satellite imagery. The goal is to better understand this tiny organism which is vital to life on earth.

### **Economic Importance**

Phytoplankton's role in the global ecosystem has made them a target for controlling carbon-dioxide levels in the earth's atmosphere. Companies such as Climos and Planktos have invested in phytoplankton as a means of reducing carbon-dioxide emissions. They are investigating fertilizing phytoplankton communities with iron, a vital nutrient, to promote their growth. As political and economic pressures to provide carbon-dioxide emissions offsets increases, the potential profit of companies like these increases.

Plankton are an important food source for organisms in an aquatic environment. They exist in oceans, lakes, rivers, and streams. Algae floating in water is a



common and easily found example of plankton. Animals rely on aquatic food sources such as algae to support the food chain.

## **Oceanic Organisms**

All organisms are separated into two classifications: heterotrophs (organisms that attain energy from other organisms) and autotrophs (organisms that obtain energy from inorganic resources, such as sunlight). Oceanic organisms are no exception. Within the oceanic ecosystem, further distinctions can be made between organisms. Both heterotrophs and autotrophs can be classified as pelagic (existing in the water column above the ocean floor) or benthic (existing on the ocean floor).

Pelagic organisms include both nekton (organisms with the ability to swim) and plankton (organisms without the ability to swim).

## **Fossil algae**

The algae are generally composed of 'soft tissue', preservation, particularly in the lower geological strata, either has not occurred or else has not been very good. Those algae that characteristically deposit lime, (e.g. Codiales, Dasycladales, Corallinaceae) or silica, (e.g. diatoms) form the groups that have been most successfully preserved and therefore about which most is known. There is little doubt that even at the present time fossilization of calcareous and siliceous algae is taking place, e.g. in the Arctic benthos where crustaceous Corallinaceae are particularly abundant. In recent years more attention has been paid to Archeozoic or preCambrian rocks and the result has been the discovery of fossil organic remains of a much greater age than previously reported.

Though there are several Late Proterozoic fossils (both unicellular and multicellular) which superficially resemble green algae, fossils which can be assigned to the group with some confidence are not known earlier than the Cambrian. This is, perhaps in large part, because the group has traditionally been defined by pigment composition, and not with more easily identified morphological characters. Fossils were thus assigned to this group because of overall resemblance to modern taxa, recent morphological work has added a number of characters which should aid in recognition of members of this group.

The **Dasycladales** has perhaps the best-known fossil record of any group of green algae. More than 120 fossil genera have been described, some dating back to Cambrian and Precambrian strata. This fossil diversity contrasts remarkably with the fact that today there are only eight extant genera, including the familiar "mermaid's cup" *Acetabularia*. The Dasycladales began to rapidly diversify in Middle Ordovician, and are common in all strata until the lower Cretaceous. Both living and extinct species are known primarily from warm marine waters,

Many members of the Dasycladales secrete lime (calcium carbonate) which increases their chances for preservation and later discovery as fossils. The group is easily recognized by their radial symmetry, with a central nonseptate axis to which are attached whorls of lateral appendages which may or may not be branched. Fossil forms are cylindrical, club-shaped, or have a spherical appearance from the density of their branches.

Many algal fossils are known and it is therefore only possible here to outline the main types of structure represented among them. Table 13.1 outlines the principal geological strata and the generally accepted time scale. Because the algae are generally composed of 'soft tissue', preservation, particularly in the lower geological strata, either has not occurred or else has not been very good. Those algae that characteristically deposit lime, (e.g. *Codiales*, *Dasycladales*, *Corallinaceae*) or silica, (e.g. diatoms) form the groups that have been most successfully preserved and therefore about which most is known. There is little doubt that even at the present time fossilization of calcareous and siliceous algae is taking place, e.g. in the Arctic benthos where crustaceous *Corallinaceae* are particularly abundant. In recent years more attention has been paid to Archeozoic or preCambrian rocks and the result has been the discovery of fossil organic remains of a much greater age than previously reported.

**CYANOPHYCEAE** The earliest known algal fossils are those from the Gunflint Chert of Ontario in rocks that are two billion years old (Barghoorn and Tyler, 1965). *Animikiea septata* would appear to have been a member of the *Oscillatoriaceae*. *Entosphaeroides* amp/us with internal spores or endogonidia could be allied to *Chamaesiphon* (p. 21) or may have been bacterial in nature. Sporelike bodies put in the genus *Huroniospora* could have allies in either the *Chroococcaceae* or the *Dinoflagellates*. In addition there are three genera of uncertain affinities, *Archaeorestis*, *Eoastrion* and *Eosphaera*. Other very early recognizable fossils are those described from late preCambrian rocks (700--900 million years of age) near Alice Springs in Australia (Barghoorn and Schopf, 1965). The specimens tentatively ascribed to the *Cyanophyceae* are small septate filaments that taper from a maximum middle zone to the ends and were possibly enclosed in a sheath very like the modern

Phormidium and Lyngbya species. All the above together with the following are best placed in a group called the Protophyceae.

**TABLE 13.1**  
**GEOLOGICAL TIME SCALE**  
(from Kulp, 1961)

ERA	PERIOD	Beginning of interval Millions years ago	
<b>CENOZOIC</b>	Quaternary	Pleistocene	1
		Pliocene	13
		Miocene	25
	Tertiary	Oligocene	36
		Eocene	58
		Pliocene	63
<b>MESOZOIC</b>	Cretaceous	135	
	Jurassic	200	
	Triassic	230	
<b>PALAEOZOIC</b>	Permian	280	
	Upper Carboniferous (Pennsylvanian)	310	
	Lower Carboniferous (Mississippian)	345	
	Devonian	405	
	Silurian	425	
	Ordovician	500	
	Cambrian	600 app.	
	Precambrian (Biotic era)	2,000	
	Precambrian (Abiotic era)	5,000	

Spongiostromata (Precambrian onwards) Much doubt has been thrown upon the authenticity of this group, some writers regarding them as structures which originated as diffusion rings ('liesegang' phenomena) in colloidal materials or perhaps in calcareous muds.

Fossil blue-green and red algae of the families Rivulariaceae and Epiphytaceae, respectively, and also blue-green algae of the genus Girvanella, were discovered in conglomeratic and arkosic beds, as well as in limestones, at both the localities mentioned above. These algae, initially, occupied pockets, hollows, and irregularities in the latePrecambrian erosion surface over which the sea advanced

early in Cambrian time, i.e. at the time of formation of the conglomerate, and they became cemented into it by trapping and binding of silt and sand and by calcification, hence building up stromatolites between the pebbles and cobbles. Within the small thickness of sediments that overlie the conglomerate, repeated, often abrupt, changes in the algal flora have been found to occur at successively higher levels. Thus, one or more species dominated the sea-floor for shorter or longer periods at different times. These changes in the flora appear to have been a response to slight variations in environmental conditions that took place from time to time during sedimentation. In the succeeding limestones (unrepresented or possibly concealed west of the southern end of Colliers Bay), only one type of alga is dominant, namely *Girvanella*, which suggests that environmental conditions were stable during the time that the limestones were being formed.

Geologically, the Late Carboniferous collision of **Laurussia** (present-day Europe and North America) into **Godwanaland** (present-day Africa and South America) produced the Appalachian mountain belt of eastern North America and the Hercynian Mountains in the United Kingdom. A further collision of Siberia and eastern Europe created the Ural Mountains.

The stratigraphy of the Lower Carboniferous can be easily distinguished from that of the Upper Carboniferous. The environment of the Lower Carboniferous in North America was heavily marine, when seas covered parts of the continents. As a result, most of the mineral found in Lower Carboniferous is **limestone**, which are composed of the remains of [crinoids](#), lime-encrusted green algae, or calcium carbonate shaped by waves. The North American Upper Carboniferous environment was alternately terrestrial and marine, with the transgression and regression of the seas caused by glaciation. These environmental conditions, with the vast amount of plant material provided by the extensive coal forests, allowed for the production of

coal. Plant material did not decay when the seas covered them and pressure and heat eventually built up over the millions of years to transform the plant material to **coal**.

The Coleochaetales are known in the fossil record only as *Parka decipiens*, shown below, which morphologically resembles some members of the living genus *Coleochaete*. Fossils of *Parka* have been found from the Upper Silurian to Lower Devonian, strata about 400 million years old. This means that they were present at about same time as the appearance of the first land plants.

Fossil specimens range from 0.5 to 7.0 centimeters in diameter. They are discoid, and apparently had a pseudoparenchymatous organization. On the surface of the disc are oval structures which may be zygotes or sporangia. They contain what have been interpreted as spores, though no **trilete mark** has been found on them.

# Macroalgae of the Ediacaran Period



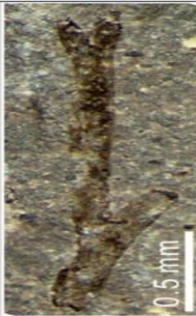
*Aggregatosphaera miaoheensis*



*Anhuiphyton lineatum*



*Anomalophyton zhangzhongyingi*



*Miaohephyton bifurcatum*



*Protoconites minor*



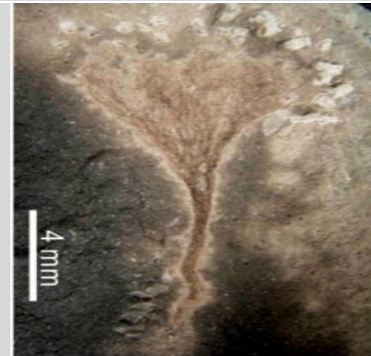
*Sinocylindra yunnanensis*



*Konglingiphyton erecta*



*Liulingjietaenia alloplecta*



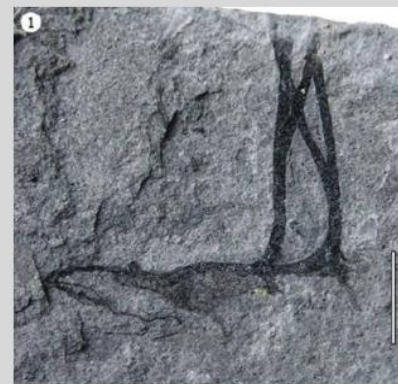
*Longifuniculum dissolutum*



*Sinospongia chenjunyuani*



*Sinospongia typica*

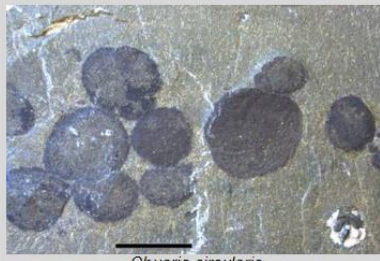


*Wenghuiphyton erecta*

# Macroalgae of the Cambrian Period



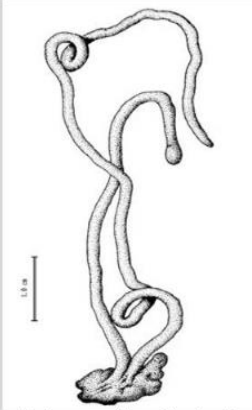
*Bosworthia simulans*



*Chuarua circularis*



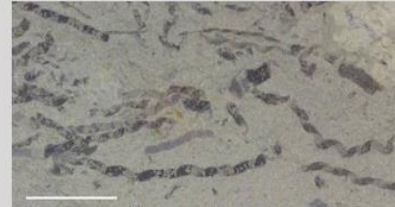
*Doushantuophyton cometa*



*Enteromorpha intestinalis*



*Fractibeltia fibrillata*



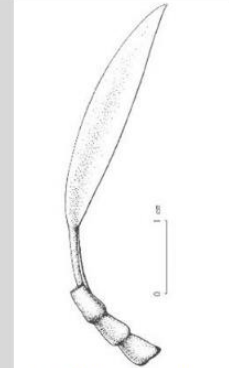
*Fuxianospira gyrata*



*Marpolia aequalis*



*Marpolia spissa*



*Paradelesseria sanguinea*



*Wahpia mimica*



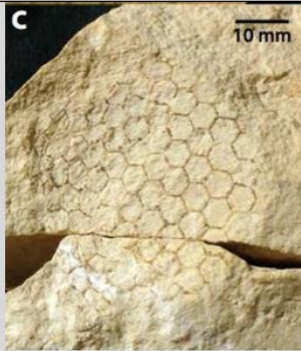
*Wahpia virgata*



*Walcottophycus gyges*



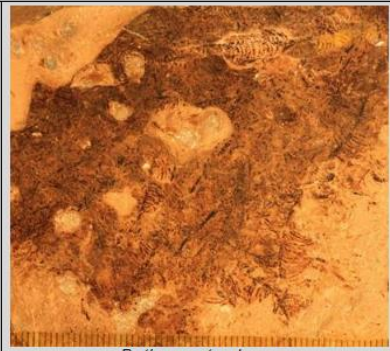
# Macroalgae of the Ordovician Period



*Amia hexagona*



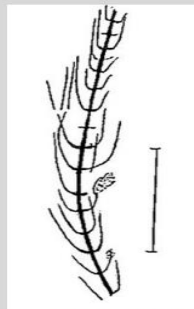
*Archaeobotaphora typa*



*Buthograptus laxus*



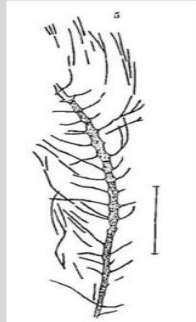
*Chaetocladus plumula*



*Chaetocladus sardesoni*



*Chaetocladus sp. 1*



*Callithamnopsis delicatula*



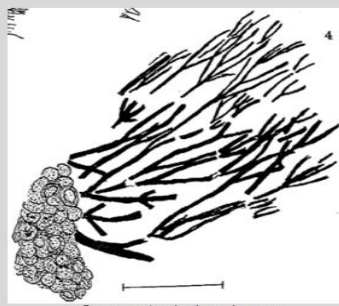
*Callithamnopsis fruticosa*



*Chaetocladus ottawensis*



*Chaetocladus sp. 2*



*Corematocladus densa*



*Dowlingia cupressina*

# Macroalgae of the Silurian Period



*Buthotrephis divaricata*



*Buthotrephis newlini*



*Callisphenus gracilis*



*Callithamnopsis silurica*



*Chaetocladius capillatus*



*Chaetocladius capitatus*



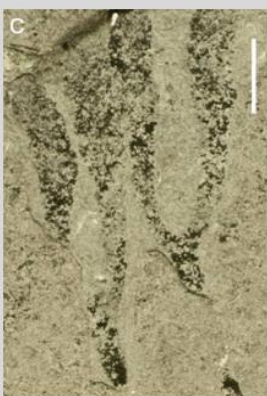
*Chaetocladius dubius*



*Chaetocladius gracilis*



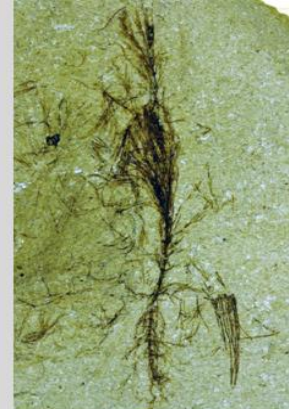
*Chaetocladius ruedemanni*



*Chondrites verus*



*Diplospirogaptus goldringae*



*Eocladius xiaoi*

## Fossil Algae

Fossil algae can be of three broad types depending on the composition of the original wall or the wall remains found in the fossil record: calcareous (in cyanophytes, coccoliths, rhodophytes, chlorophytes and charophytes); siliceous (in diatoms and chrysophytes); and organic (in acritarchs, dinoflagellates, prasinophytes and certain chlorophytes). Table 23 summarises the main groups of fossil algae, their characteristics and geological range.

### Calcified remains

#### *Cyanophyceae*

Fossil cyanophytes (so-called 'blue-green algae') are known from the Archaean to the Quaternary. Archaean fossils include stromatolites and kerogen or complex biologically-produced molecules of carbon (Walter, 1976). Stromatolite formation usually involves coccoid or filamentous Cyanophyceae; indeed there can be marked similarities between the fossil taxa and their modern counterparts (Fig. 16). The mucilaginous layer that covers the living cells binds mineral particles to form laminae. In some exceptional cases, where the structures have been silicified to form a chert, it is possible to recognise individual micro-organisms, but usually the only records of past life are the laminae. Studies by Schopf (1992a, 1992b, 1994), Schopf & Walter (1983), Hayes (1983, 1994), Hill *et al.* (2000) and Walter (1976, 1983, 1994) documented the systematics and geochemistry of the first records of life on Earth.

Archaean stromatolites have been found in Australia, southern Africa and Canada. The Palaeoarchaeon (3.8–3.2 Ga) is well represented by the Onverwacht Group, South Africa and the Warrawoona Group of Western Australia. These localities, with an age of 3.55 Ga, include filamentous bacteria and cyanophytes. The stromatolites from the Warrawoona Group are stratified, pseudocolumnar and dome-shape (Walter, 1994; Fig. 16). The Insuzi Group from South Africa, with benthic taxa (Walter, 1983), are good examples of stromatolites from the Mesoarchaeon (3.2–2.8 Ga), while excellent Neoproterozoic sites (2.8–2.5 Ga) have been found in the Fortescue Group, Western Australia (Buick, 1992) and the Campbell and Ventersdorp Groups, South Africa, with some filamentous cyanophytes similar to *Lyngbya* (Walter, 1998).

The Palaeoproterozoic Gunflint Formation (1.8–2.1 Ga) of Ontario, Canada, shows excellent preservation of unicellular and filamentous cyanophytes (Taylor & Taylor, 1993). In Australia, Grey (1994a, 1994b) described new taxa of stromatolites from the Earraheedy Group in the Earraheedy (formerly Nabberu) Basin and the Glengarry Group, both in Western Australia, some of the forms being useful as stratigraphic indicators. Walter *et al.* (1988) described assemblages from the McArthur and Georgina Basins and the Mount Isa Province, Northern Territory and Queensland. The Georgina Basin is Late Proterozoic (in its oldest part), whereas the other areas are Mesoproterozoic in age.